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13. ABSTRACT (Maximum 200 Words)

Neurofibromatosis Type 1 (NF-1) is the most common autosomal dominant genetic disorder, affecting the skin, central (CNS) and peripheral nervous systems. Children with NF-1 have an increased risk of developing significant learning disability (LD), cognitive impairment, and optic or brain stem gliomas. Cerebral magnetic resonance imaging (MRI) in NF-1 reveals regions of high signal intensity (often called "unidentified bright objects", or UBOs). The pathophysiology of UBOs is poorly understood, and it is controversial to what extent they are involved in cognitive impairment. The aims of this proposal are to characterize the underlying metabolic abnormalities in NF-1 with proton MR spectroscopic imaging (MRSI). We have developed a rapid, quantitative MR spectroscopic imaging (MRSI) protocol for the evaluation of cerebral metabolite levels in NF-1. Metabolite levels will be determined both in UBOs and other brain regions, both in order to improve understanding of the etiology of UBOs, and to understand the relationship between regional brain metabolism and LD. 60 subjects with NF1 and 60 control subjects will be evaluated with proton MRSI and detailed neuropsychological testing. Ultimately, proton MRSI may be a useful test for identifying children with NF-1 at risk of developing LD, and also help in distinguishing UBOs from other, malignant lesions which require therapeutic intervention.

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Introduction

Neurofibromatosis Type 1 (NF-1) is the most common autosomal dominant genetic disorder, affecting the skin, central (CNS) and peripheral nervous systems. Children with NF-1 have an increased risk of developing significant learning disability (LD), cognitive impairment, and optic or brain stem gliomas. Cerebral T_2-weighted magnetic resonance imaging (MRI) in NF-1 reveals regions of high signal intensity (often called "unidentified bright objects", or UBOs) in the basal ganglia, brain stem and cerebellum. The pathophysiology of UBOs is poorly understood, and it is controversial to what extent they are involved in cognitive impairment. Proton magnetic resonance spectroscopic imaging (MRSI) is a relatively new non-invasive metabolic imaging technique that can provide information about the cellular composition and metabolism of brain tissue. Our pilot data of proton MRSI in NF-1 indicate highly significant perturbations in thalamic metabolism in NF-1, regardless of presence or absence of UBOs. UBOs themselves were metabolically more similar to normal brain tissue. These data indicate dissociation between imaging and metabolic findings, and may indicate more widespread cerebral involvement in NF-1 than that indicated by MRI.

In this proposal, we are extending these preliminary findings to investigate the hypotheses that: (1) thalamic metabolism is abnormal in NF-1 and evolves with age, (2) proton MRSI measures of thalamic metabolism will correlate with neuropsychological performance, and (3) metabolic abnormalities in NF-1 are more diffuse and widespread than abnormalities visualized by MRI. The study design to test these hypotheses involves the performance of proton MRSI, MRI and neuropsychological testing in 60 subjects with NF-1 and 60 age-matched control subjects. To test hypothesis (1), thalamic metabolite levels will be compared between NF-1 subjects and controls in 3 different age ranges, and regression analysis performed with respect to age. To test hypothesis (2) thalamic metabolite levels in NF-1 patients will be correlated with results of a battery of neuropsychological tests. To test hypothesis (3), multiple regions of interest in the basal ganglia and cerebellum will be evaluated both by MRI and MRSI, and compared between NF-1 and control subjects.

In addition to improving the understanding of the pathophysiology of NF-1 brain lesions, this proposal will establish the relationship between regional cerebral metabolism and cognitive impairment in NF-1. If successful, MRSI may serve as a screening tool for young children with NF-1; the observation of normal MRSI may be reassuring prognostic information for normal subsequent development, while children with abnormal MRSI may be identified for early intervention for possible learning or developmental problems.

Body

After protocol development and optimization of study design in year 1, in year 2 we have been actively involved in patient recruitment and entering of subjects into the research protocol. So far, 17 patients and 16 control subjects have been studied.

We are interested in evaluating the biochemical composition of UBOs as a function of age, as well as the examination of adjacent brain parenchyma in NF-1, as well as cerebral metabolism in patients without UBOs. We have examined a well-characterized group of subjects with a range of phenotypic expression of NF-1, and age- and sex-matched control subjects. Based on postmortem and imaging data described above, we hypothesized that the pathologic process underlying UBOs is relatively diffuse in nature, therefore, may be underestimated by conventional MRI. We circumscribed our measurements to supratentorial regions, considering that these locations consistently demonstrate UBOs with an age-dependent involution.

Nine male NF-1 patients (age range, 6-19 years; median, 12 years), 4 with normal MRI, and 5 with UBOs, which were participants in a research program aiming at characterizing the neurobehavioral phenotype of NF-1, constituted the experimental sample. All subjects were seizure-free and only one, in the subset with UBOs, had a small optic pathway glioma. So far, only the neuroimaging data and MRSI has been evaluated in these cases. The control group consisted of 9 sex-matched subjects (age range, 6-19; median, 14 years) without any history of neurological disease and with normal brain MRI. All subjects were examined with conventional MRI and quantitative, multi-slice MRSI (for details, see methods section).

All subjects that, in the present sample, showed supratentorial UBOs had these abnormalities predominantly in the globus pallidus/internal capsule region. Accordingly, on one slice of the MRSI data set, regions of interest were identified in the thalamus, basal ganglia, and occipital gray matter. The latter region, which is typically uninvolved by hyperintense foci, served as an internal control. At least 2 pixels were identified which were completely encompassed by each structure, and each structure was measured in both left and right hemispheres. Since no left-right asymmetry could be identified, data from left and right hemispheres were averaged together before further statistical analysis. For each region of interest, absolute metabolite concentrations were calculated using the phantom replacement method (see methods section) which we have previously developed and validated. Metabolite ratios were also calculated for comparison with other, non-quantitative studies. Ratios are also insensitive to partial volume effects with CSF and potential systematic quantitation errors, and in some circumstances (e.g. when Cho increases and NAA decreases) ratios may be more sensitive (i.e. the NAA/Cho ratio) to detecting small changes than measurements of either metabolite concentration alone. For visual

screening of MRSI data, metabolic images of individual metabolites were also recreated (Cho, 3.34-3.14 ppm; Cr, 3.14-2.94 ppm; NAA, 2.22-1.82 ppm; lactate, (not detected) 1.55-1.15 ppm).

Descriptive statistics consisted of calculation of means and standard deviations of metabolite concentrations and ratios. Due to the small sample size, metabolite concentrations and ratios were then compared between conditions using non parametric tests. We specifically used a variation of the Wilcoxon test, termed Robust Rank Order test (55). Both one- and two-tailed tests were applied. The latter does not assume a particular direction of the changes experienced by the experimental group, whereas the former applies to situations with predictable outcome. In this particular study, we hypothesized that the changes in metabolites will include decreases in NAA, due to white matter edema and/or neuronal injury, and increases in Cho as a consequence of high turnover of myelinic membranes and/or gliosis. Therefore, one-tailed distributions of measurements were also considered. Data and statistical analyses were done using the Statview 5.0™ program.

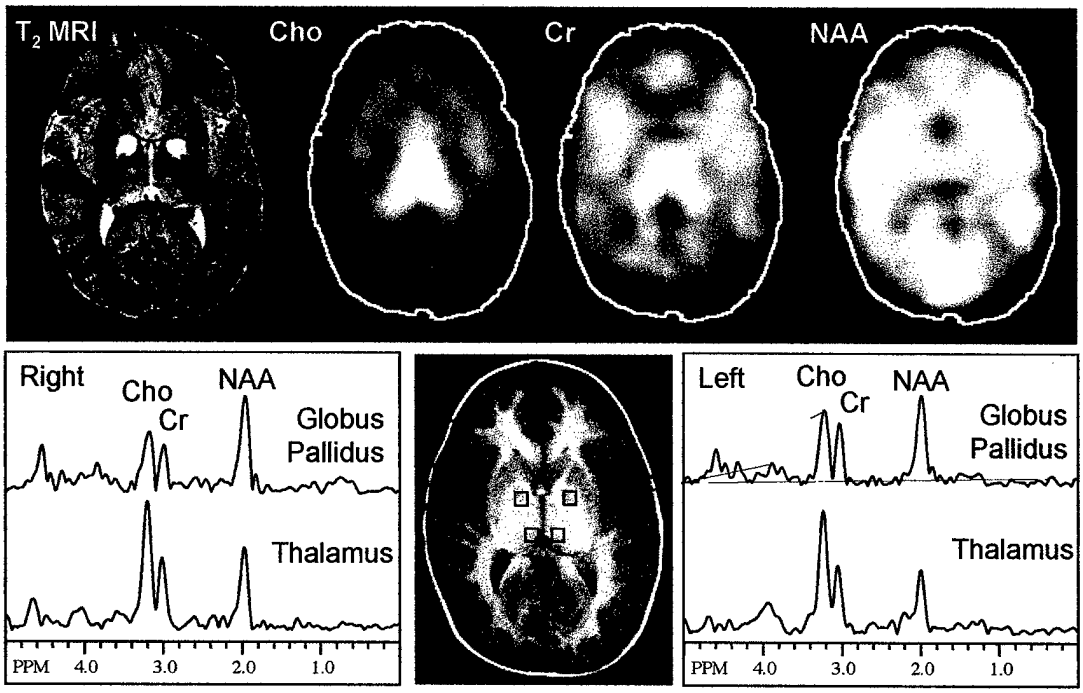

Figure 1. T1, T2 MRI and proton MRSI in an 8 year old NF-1 patient with prominent UBOs in globus pallidus/internal capsule and subtle thalamic UBOs. Metabolic images and spectra from both thalami show large increases in choline, while UBOs (in the globus pallidus) are relatively normal.

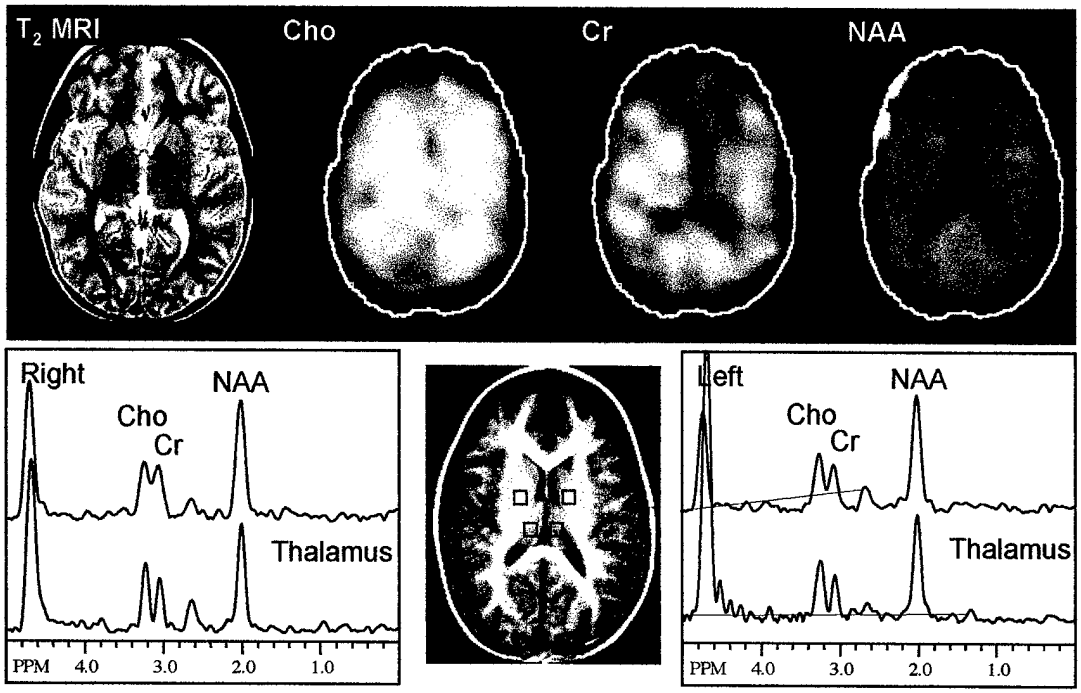

Figure 2. T1, T2 MRI and proton MRSI in an 8 year old control subject.

Figure 1 shows an example of MRI and proton MRSI in an 8 year old subject with prominent UBOs in the globus pallidus and subtle thalamic UBOs. MRSI reveals high levels of Cho primarily in the thalamus, with more normal appearing spectra from the pallidal UBOs. For comparison, spectra and images from an 8 year old control subject are shown in Figure 2. Since qualitative evaluation of the data sets indicated abnormal metabolism in the thalamus, in addition to evaluating UBOs, we also quantitatively analyzed spectra from the thalamus and occipital gray matter (considered to be an uninvolved "control" region") in all subjects. Results of the quantitative analysis (absolute metabolite concentrations and ratios) for all subjects are given in Table 1.

TABLE 1. Metabolite concentrations (mM) and ratios in thalamus, globus pallidus (including UBOs, if present), and medial occipital gray matter (GM) in NF-1 and control subjects. Values highlighted in bold are significantly different ($\alpha < 0.05$). *Indicates NF-1 patients without UBOs in globus pallidus. **Indicates thalamic measurements in NF-1 patients with UBO in globus pallidus. Note that only 4 out of 9 control subjects had interpretable spectra from the globus pallidus.

			[Cho]	[Cr]	[NAA]	NAA/Cho	NAA/Cr	Cho/Cr
Thalamus	**NF-1**	Mean	2.56	6.47	**5.64**	**1.04**	**1.74**	1.74
	(N=9)	St Dev	0.71	1.31	0.99	0.20	0.29	0.39
	Controls	Mean	1.95	6.04	**7.45**	**1.71**	**2.52**	1.50
	(N=9)	St Dev	0.45	1.70	1.75	0.19	0.17	0.19

	NF-1*	Mean	2.28	6.05	5.94	**1.19**	**1.95**	1.69
	(N=4)	St Dev	0.31	0.95	0.93	0.12	0.09	0.17
	UBO**	Mean	2.78	6.81	5.40	**0.92**	**1.57**	1.78
	(N=5)	St Dev	0.89	1.56	1.09	0.16	0.29	0.53
Globus Pallidus	**NF-1***	Mean	1.61	5.13	6.53	1.86	2.56	1.48
	(N=4)	St Dev	0.31	0.87	0.50	0.22	0.65	0.48
	UBO	Mean	1.85	5.85	5.84	1.49	2.00	1.41
	(N=5)	St Dev	0.58	1.77	1.18	0.41	0.39	0.41
	Controls	Mean	1.72	5.00	6.20	1.71	2.58	1.60
	(N=4)	St Dev	0.46	0.82	0.34	0.41	0.53	0.38
Occipital GM	**NF-1**	Mean	1.38	**5.33**	7.09	2.24	2.58	1.16
	(N=9)	St Dev	0.41	1.15	1.93	0.36	0.57	0.24
	Controls	Mean	1.68	**6.68**	8.18	2.26	2.47	1.13
	(N=9)	St Dev	0.39	1.04	1.53	0.33	0.31	0.28

Key Research Accomplishments

- Established MRSI and neuropsychological test methodology
- Collected data so far in 17 NF1 and 16 control subjects
- The demonstration of abnormal, age-dependent thalamic metabolism in children with NF-1

Reportable Outcomes

Since we are in the data collection phase of the project, there have been no publications this year regarding results in NF1.

Conclusions

The collection of data for the evaluation of the relationship between neurometabolism, in particular thalamic NAA and choline levels (and the ratio of NAA/Cho), and LD in NF-1 as described in the proposal is continuing. Year 3 will enlarge significantly the number of subjects . Advertisements for subject recruitment have been placed with the Neurofibromatosis Society which we believe will increase the referral rate for subjects with NF1 .

This work is important for the clinical evaluation of patients with NF-1 in two respects. Firstly, proton MRSI may allow for a quantitative biochemical determination of the degree of brain involvement in children with NF-1, the observation of normal MRSI may be reassuring prognostic information for normal subsequent development, while children with abnormal MRSI may be identified for early intervention for possible learning or developmental problems. Secondly, the characterization of UBO metabolism is important for the diagnostic reasons; since patients with NF-1 are at increased risk for development of brain and optic gliomas, it can sometimes be difficult to distinguish these very different pathologies using conventional magnetic resonance imaging. Early, non-invasive diagnosis of a malignant glioma (and distinguishing it from a benign UBO) is extremely important in improving therapeutic outcome inthese patients.

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Appendices

None